



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

FURTHER STUDIES IN VOLVOX, WITH DESCRIPTIONS OF THREE NEW SPECIES

BY J. H. POWERS

WITH FOUR PLATES

In the last volume of the TRANSACTIONS the writer published an article on "New Forms of Volvox," in which two hitherto unrecognized types were described. Each differed radically from all previously known species. But as the description of each rested upon but a single collection of material and as their nearest ally—*Volvox aureus*—is known to be a highly variable species, it was deemed unwarranted to bestow upon them new specific names until further investigations of material from wider areas had been made to throw light on possible connecting forms. Such further studies have now been made. A number of valuable collections of *Volvox* have been secured, from points, in some instances, as widely separated as Maine, Washington and Louisiana; while continued effort in the writer's own state (Nebraska) resulted during the past summer in finding the organism in nearly a score of bodies of water. Many of these were insignificant in size and duration. But, in the full blaze of Nebraska sunlight, *Volvox* is able to appear, multiply, and riot in sexual reproduction in pools of rain water of scarcely a fortnight's duration. Indeed, the ecology of the organism deviates here decidedly from that which has usually been assigned to it. Upon another occasion the writer may describe this more in detail. Suffice to say now that the writer's repeated failure to find *Volvox* in previous years has probably been due to seeking for it in too large bodies of water, *e. g.*, ponds one to two feet deep. Probably a hundred of these have again been examined during the past summer without success in a single instance.

The amount of material secured from all sources has been very considerable. Nearly a hundred bottles stand on my shelves, many of them containing many cubic centimeters of *Volvox* alone. A brief indication of the results thus far obtained may be given in

few words. First, the hope that a form had been discovered in the process of extensive variation has not been substantiated. Second, on the contrary, the "first form" described by me in the previous paper proves to be a new and definite species. Third, as to the "second form" described, nothing resembling it has again been found. As above stated, no *Volvox* has again been met with in deeper ponds resembling the one from which this form was taken. Fourth, the surprising fact has developed that at least two other distinct species exist in our fauna, displaying varied and interesting characters which separate them distinctly from the accepted European species as well as from those which I have described. Fifth, no less surprising is the almost total absence, from the material I have examined, of the European types. In three instances only have isolated colonies been met with showing feeble sexual reproduction of the European type of *V. aureus*. Not a trace of the true *V. globator* in sexual reproduction has yet been found. But a beautiful form resembling *V. globator* in its somatic cells I possess in copious quantities. It has undoubtedly been mistaken for *V. globator* in repeated instances. But measurements and, above all, the study of its sexual phases at once reveal it as distinct. Indeed, it is not only a separate species but, again, a surprisingly aberrant form, extending in yet a new respect the limits of generic characters. It will be seen at the close of this paper that the genus *Volvox* includes diversities, both on the sexual and somatic side, that would better become a family than a genus. Yet its division into separate genera is plainly out of the question because of the contradictory nature of these two classes of equally fundamental characters.

In the remainder of this paper I wish, first, to name, refigure and complete the description of the "first form" of *Volvox* described in my preceding publication; second, to figure and describe in detail a second interesting species, discussing in connection with it a remarkable process of invagination and final inversion of young colonies which it displays more frequently than any other species. Third, I will briefly describe, without figures, the third new species; adding, finally, a few words of theoretical discussion.

VOLVOX SPERMATOSPHERA

The first form of *Volvox* described by me in the above mentioned

paper I now name *Volvox spermatosphara*, the allusion being to the "sperm spheres" which constitute its most obvious and interesting character. It is not necessary to re-describe this species in detail as this has been sufficiently done in the previous paper. The figures there given also represent many aspects of the species correctly, although the general views of entire coenobia were so imperfect, owing to the inadequate material at my disposal, that I have bettered and extended them by the figures on plate XXIII; while figures 14 to 22 and also figure 26 on plate XXIV show more aberrant coenobia and certain special details in regard to reproduction. It remains at present to give evidence for the specific distinctness, to modify one or two partial errors, to confirm several doubtful points, and to add such new details as the study of the living material has disclosed.

As to the independence of the species, it proves, in most respects, to be quite constant. By persistent search, I was able to collect a large amount of the material during the months of July, August, and September, from quite a number of distinct pools. I observed the species both in its appearance and disappearance, although none of the locations allowed me to follow it for many weeks at a time. But all phases of its life history were observed time and again, without noting other deviations than those in size, etc., to be mentioned later. The same uniformity was noted in collections of material submitted to me from two or three distant points. The first of these, for which I am much indebted to Dr. Elda R. Walker, whose article on "Observations on the Micro-Fauna of an Oregon Pond" appears in this volume of the TRANSACTIONS, was from Washington. Although sexual phases were not abundant, sufficient were present to completely prove its identity with the form here described. The second was an invaluable and copious collection of *Volvox*¹ kindly submitted to me by Prof. R. H. Wolcott, of the

¹This remarkable collection contained not only examples of the entire three species here described, besides two instances of decidedly variant coenobia which may very possibly belong to a fourth species, but it also contained, in lesser quantity, a wide range of species from other genera of the Volvocineæ. Among these latter were numerous individuals, in all stages of growth and vegetative reproduction, of the beautiful organism *Pleodorina californica* Shaw. I have also seen this organism in the collections of E. Foster of New

University of Nebraska. It was taken in a broad but shallow pond near Rocheport, Missouri, on July 24, 1904. The water of the pond was said to be decidedly warm to the touch when the collection was made. *V. spermatozophara* comprised more than half the collection, showing every phase of the organism's development in countless numbers. It is from this collection that the additional figures above mentioned were taken. As above stated the evidence from this locality also showed the species to be a fairly constant type. *Volvox* colonies found in plankton collections kindly loaned by E. Foster of New Orleans showed the probable presence of the species there, though the sexual forms were not found. It will thus be seen that the localities for the species, thus far, are all from western America. Yet it can scarcely be restricted to this area.

The first point of my previous description that requires modification is the matter of size. I there gave the limits of 500 to 1,000 μ for the diameter of the colonies. Although the best estimates I could make, and certainly not far from correct for the material then at my disposal, I find they greatly exaggerated the size of the species as I have since found it. All, indeed, of the material since examined by me has averaged small. This may no doubt be due to the circumstances of its growth, *viz.*, little water and much crowding, but the first and only material seen by me when I wrote the original description was doubtless quite as exceptional or more exceptional than the larger quantity examined since. The species averages small, perhaps smaller than any other in the genus. My more recent measurements show mature reproductive coenobia of vegetative and mixed content reaching a common maximum of 600 to 650 μ . Purely female colonies reach but about 500 μ , while the smallest showing mature sperm spheres are at times as small as 150 μ or even smaller. The most frequent size of mature coenobia in the above mentioned larger collection from Missouri

Orleans. Hitherto this *Pleodorina* has to my knowledge, been recorded only from California and Illinois. I may also here take occasion to correct an oversight touching *Pleodorina* in my last article. I there spoke of observing transition types between *Pleodorina* and *Eudorina*. I should have said *Pleodorina illinoiensis*. This species and this species only is a variety of *Eudorina elegans*.

was not far from 350 μ in average diameter. These dimensions illustrate the extreme variability in size of the species, paralleling in this respect *V. aureus* which, according to Klein, varies from about 170 to 850 μ . The colonies shown on plate 1 will display these dimensions approximately to the eye. All are photographed under the same magnification (about 79 diameters) but it should be borne in mind that the flattening necessary for photography has not always been in equal degree, while in any case, some coenobia crush rather than expand in the process. The relations are therefore approximate, not exact. All the figures on plate XXIII except figure 3, represent nearly or quite adult colonies, and all are taken from the same collection of material. The minimum size of free young colonies also falls far below that found in the material first described. They frequently escape from the parental coenobia at 100 μ or less (fig. 3).

Observation of living material emphasizes another minor though constant character, *viz.*, the uniformly oval shape of the coenobia at all ages. In no other species of the genus, if we may trust descriptions and figures, is this character so strongly developed. Even before birth it is strongly marked (figs. 1 and 2), while in *V. aureus* the young are said to be spherical. This character is no doubt correlated with the unusually active habit and extraordinary development of polarity which this species manifests. To these I will return later.

As to the general nature of the various reproductive contents of the colonies, most of the facts remain as previously described. The decreased average size of the colonies reduces somewhat the average number of reproductive bodies occurring, though by no means proportionately. This is especially noticeable in the numerous smaller female colonies (pl. XXIII, figs. 9 and 10). These show a marked tendency to contain exactly eight ova; but frequently this number is increased to eleven, twelve, or more; while large female colonies, like figure 4, although they now and then contain many more, usually do not do so. The ova or oosperms in tiny colonies like figure 9 often well nigh fill the entire interior.

Similar to this last phenomenon, but much more striking, is the frequent overcrowding of parent colonies, both rather large as

well as very small ones, with asexual daughter colonies or with these and sperm spheres (pl. xxiv, figs. 20, 23, and 26). No such hypertrophied reproductive development has, to my knowledge, been hitherto recorded for the genus. All the figures I have seen have shown the daughter colonies as fairly roomily situated within the parent. When first found, I was inclined to attribute the extraordinary appearance to some unwonted shrinking action of the preservative. But I have since verified the exact appearances on living material. As will be seen from the figures, the parent colony is often stretched by the growing young until it becomes a mere taut film assuming any form which the chance aggregation of the progeny requires. Not only is the gelatinous wall of the parent colony, normally of considerable thickness in this species, reduced to attenuation, but even the individual cells of the parent colony, where the pressure of the growing young is greatest, are flattened out until their longer, longitudinal axis has become much shorter than the transverse. An interesting result of this overcrowding is the frequent crushing of the more mature sperm spheres when these are present (pl. xxiv, fig. 26). The ordinary daughter colonies prove very resistant, showing but now and then a little distortion; but the spheres of sperm platelets succumb at once to the pressure, another significant proof of their non-independent character. As they mature, they are, indeed, most frequently flattened against the inner surface of the colony producing them. And this, again, I find to be not an artefact, but present in living material.

Touching this same matter of the independence or dependence of these cell aggregates which resolve themselves wholly into sperms within the mother colony, I have made some further observations upon the living material. It is obvious that the sperm spheres when made up of mature sperm platelets cannot be independent locomotive bodies. The cilia of the separate sperm bundles do not extend freely into the surrounding medium but are quite confined within the thick, if fragile, envelope of the entire sphere. Somatic cells are not present whose flagella might serve for common locomotion. However as sexually mature *Volvox* colonies, even when free, usually tend to quiescence, we might still suppose these spheres

capable of a free life and of locomotion in their earlier stages. Moreover, I have again verified upon living material the somewhat uncertain observation first made on the original slides to the effect that the sperm spheres possess rudimentary cilia in the spermogonia stage. I was, however, unable to find a single free sperm sphere among living material rich in sexual colonies. I therefore tried the experiment of freeing them by artificial rupture of the parent. I chose coenobia carrying both sperm spheres and the ordinary daughters, as these latter would furnish an obvious control in the results. In every instance, when thus freed, the ordinary daughter colonies showed the utmost celerity in locomotion. They seemed to me the most rapidly moving *Volvox* aggregates I had ever seen. But the sperm spheres were powerless to change their location. Those slightly less mature were quite inert, while a few, evidently in about the last stages of development preceding the final divisions, showed an interesting rudimentary power of movement. Without changing their location they rotated, with a slow continuous movement, about an axis vertical to the substratum. The movement was so slow as to be but just perceptible under low magnification. It was continued, however, as long as I watched them, for perhaps a quarter of an hour. Unfortunately time did not permit me to experiment upon the possible development of these artificially freed groups of purely sexual cells. Such experiments could not fail to be of interest. The above observations are sufficient, however, to establish the thoroughly dependent nature of these aggregates. If they are reduced *Volvox* colonies, they are, as I have stated in my earlier article, devoid of the majority of the characters of such. They stand to the parental colony wholly on the plane of a spermary or antheridium. I shall return to a few words of a theoretical nature touching these at the close of the article, showing that quite a different hypothesis may be made with regard to their origin. I may remark here, however, that a few new observations have been made upon the size of these sperm spheres. In my preceding description I gave the numbers of sperm platelets as 64, 128, or 256, allowance being made for irregularities due to the occasional degeneration of cells. I find, however, in the very small coenobia, frequent in many of my later collections, that the number of platelets in mature sperm

spheres is often but 32 (pls. xxiii and xxiv, figs. 11, 12, 13, and 14). If, then, the final interpretation put upon these bodies makes them true colonies, we must begin our definition of the genus, not with, "cells numerous, usually above a thousand, etc." but "number of cells various, from 32 to at least 22,000."¹ However, a word later in this matter, after the next species has been described, for it, again, has a related, though less extreme, mode of sperm production, which will cast considerable light upon the matter of interpretation.

As to the number and size of the individual sperms, there is more variation in the latter than I at first noted, but they are fairly constant and I have not made further measurements. The number in the platelet appears, too, more constant than in any other species of the genus; but among many thousands observed I have seen perhaps half a score of cases where the number was 64 instead of the usual 32. Such is the case with the sperm bundles in the mature and flattened sperm sphere shown in pl. xxiv, fig. 16. All the platelets evidently preserve a uniform number in these rare deviations, just as they invariably do in the ordinary type.

In connection with the formation of sperms, I have sought assiduously for transitional variations between *V. spermatosphara* and *V. aureus* or *V. tertius*. These would naturally appear, either as adult colonies developing at least a few sperm bundles in the ordinary isolated or scattered fashion; or, second, as young precocious male colonies midway between sperm spheres and the precocious progeny which Klein described in the rare variant of *V. aureus* and which Meyer found in *V. tertius*. As to the former, not an adult colony of *V. spermatosphara* has shown so much as one sperm bundle in the position occupied by them in the two European species, more especially in *V. aureus*. It is true that I collected a few colonies of a small *Volvox* from a spring-fed pool in the sand-hill district of western Nebraska, among which I at last discovered one or two sexually mature male coenobia that conformed wholly to the type of *V. aureus*. But as none of them showed definite characters, so far as my investigation could be carried, of

¹The maximum is given by Klein for *V. globator*. It is quite possible that the last species to be described in this paper, which far exceeds *V. globator* in size, may also exceed it in number of cells, etc.

V. spermatosphara, I conclude that these colonies probably were the true European species. It is the only certain instance of it that has thus far fallen under my observation.

As to the second possible line of connection, a search of thousands of coenobia has shown me two, which may be doubtfully regarded as such connecting types. They were from the Missouri collection which was so exceedingly rich in varied forms of *Volvox* and related genera. But in each instance it appeared rather more probable that they were pathological colonies than either true variants, or yet examples of another special species. They were small coenobia, each showing injury and rather weak somatic cells. But the point in question was that each bore within it, daughter colonies made up largely of sperm platelets, but with a few undivided and therefore possible somatic cells. Figure 24 on plate XXIV represents one of these daughter colonies, under a magnification of 217 diameters. The sperms were mature, but will be seen to be exceedingly small, much smaller than any that I have met with in typical *V. spermatosphara*; and, besides this, there were in every case, but sixteen in a bundle, a number common in typical *V. aureus*, but never found by me in the special species under consideration. These two isolated instances of possible yet improbable connecting links demand no more attention until their nature has been demonstrated by the discovery of more material of a similar type.

There remains to be discussed several points of interest concerning the structure of the individual cells of the coenobia. In the *American Naturalist* for 1889, John A. Ryder described a so-called "anterior sense organ" in a form of *Volvox* identified by him as *Volvox aureus*. The fact discovered—as interesting as the designation was unhappy—related to the development of the stigmas. Those at the anterior pole proved to be "eight or ten times" as strongly developed as those at the posterior pole. It is curious that this interesting discovery has not, to my knowledge, been made, or even repeated by other observers, European or American. Possibly the same species may or may not display the character according to the environment (character of water; amount of sunlight) which it is developed. This was my thought when, upon examining my first *V. spermatosphara*, taken from a decidedly muddy pool,

I found its stigmas powerfully developed in the cells of the forward pole of each coenobium, yet regularly decreasing in size, until, a little posterior to the equator of the colony, they were no longer present, or at least no longer present as colored bodies. I was unable to decide whether at the posterior pole, they were really quite absent, or whether one of a group of small colorless granules really was the homologue of a pigment body. However, I could find no coenobium which showed recognizable stigmas at the posterior pole, even when well viewed under immersion lenses. Even in daughter colonies before birth the differentiation is strongly marked. The stigmas are visible, even under low magnification, at the anterior poles, but in each instance, I failed to follow them, despite most careful search, beyond a point a little past the equatorial plane. These facts point to the peculiarity as most probably a constant specific character, rather than a local adaptation only, for the young colonies are by no means regularly so placed in the parental coenobium that the anterior pole only is illuminated. It is probable that this extreme differentiation is correlated with the unusual degree of polarity in this species, with its habit as a very active swimmer, and with its ability to withstand decidedly muddy water. Further study must determine whether this peculiarity is present in other species as well. Unfortunately conclusions cannot be drawn from preserved material, as the stigmas are not conserved.

Finally, a word concerning the important feature of the cytoplasmic strands or processes supposed to extend from cell to cell of the *Volvox* colony. Much debated for *V. globator* and finally definitely established by Arthur Meyer, they seem also invariably present in *V. aureus*, though delicate, straight and as fine as the cilia throughout their entire length. In *V. tertius*, too, Meyer found them in the younger colonies, but, despite his long research and elaborate technic upon this particular point, they quite escaped his observation in the older colonies. Still he doubted their absence. I have carefully repeated his technic, using the same Zeiss lenses which he employed. I have also used other and newer reagents which seemed indicated for the purpose in hand. But in this species of *Volvox* I detect not the smallest trace of connectives in colonies of any age whatever. If this finding is borne out by the observa-

tions of others, it seems gratuitous to assume the presence of such connection between cells as a generic character of *Volvox*. I may add that the reagents employed brought out with much plainness the minutest features hitherto observed in other species. The demonstration of the finest fibrils which Meyer showed to be the true connectives uniting the larger processes, cell with cell, in *V. globator*, gave me no difficulty. Cilia were plain, even in living daughter colonies, viewed through the thick matrix of the parent. I think I should have discovered the connectives easily had they been present at one-fifth the diameter of the cilia.

As to the structure of the gelatinous sheaths surrounding the individual cells, I have, following Meyer, made it out fully, using his reagents on fresh material and also by very prolonged staining of preserved material in carmalum. I regret that lack of time prevents my preparing figures of this feature for present publication. My preparations, however, show the present species closest in this respect to *V. tertius*, though distinct from it. Most noticeably so in the marked polarity of the sheath. The thickness of the matrix about the posterior cells is several times that of those at the anterior pole.

Summing together the more important characters for the species *Volvox spermatosphara*, we get the following definition:

Coenobia strongly oval, the sexually mature members from 150 to nearly 1,000 μ in diameter, more commonly between 250 and 600 μ , containing approximately 1,000 to 3,000 cells. Polarity and activity of the coenobia strongly developed, the single cells showing stigmas decreasing regularly from the anterior toward the posterior pole. Form of individual cells spherical to oval in side view, without connecting strands of cytoplasm. Numbers of primary sex cells approximately the same, whether becoming parthenogenetic daughter colonies, ova, or aggregates of male cells (spermogonia)—from 1 to 25, or perhaps more. Ripe sperms in bundles of 32, situated, not in the matrix of independent colonies, but in aggregates within the parental colony—sperm spheres—each of which contains from 32 to approximately 256 bundles.

VOLVOX WEISMANNIA

While studying the beautiful collection of material collected by Professor Wolcott at Rocheport, Missouri, I met with numerous coenobia of a *Volvox* quite unknown to me which at first glance I thought might be a variant of *V. spermatosphara* or possibly a link between it and some other type. A little study, however, showed it to be wholly distinct from *V. spermatosphara* and from all other species hitherto described. It is indeed one of the most unique species of the genus. Not only does it display several very exceptional and yet unvarying characters, but it shows its specific distinctness still more conclusively by the large number of constant minor characters which separate it from other described species. In order to facilitate to the utmost recognition of the species, which I am especially desirous should be restudied in other developmental stages and in other localities, I will describe these characters in the order of their significance and of their obviousness, rather than in the conventional series.

Perhaps the most striking and certainly the most fundamental of these characters is the early period at which the primary reproductive cells are set apart as distinct from those which are to become the vegetative or somatic members of the adult aggregate. The ten smaller figures in the upper half of plate xxvi make this fairly plain. All are photographed with a magnification of 217 diameters and all are from decidedly young, though not equally young, coenobia. Several are taken just before the time of closure (figs. 50 to 54), while others (figs. 45, 47, 48, 49, and 56) in stages just subsequent to this event. Perhaps most characteristic and instructive of all are figures 51 and 52. These are taken from the two opposite poles of two similar daughters in the same coenobium. Young coenobia in exactly this stage and showing exactly this appearance were present by hundreds in the material. It will be seen that the young colony is still quite unclosed, yet the reproductive cells are not only distinct and well developed but they have already quite lost their connection with the periphery of the colony, lying merely in delicate contact with the inner ends of the somatic cells which are now evenly distributed above them. Figure 53 shows, poorly, an earlier stage, while figures 50 and 54 were intended to show

yet earlier stages, the number of cells still being small, although no less certainly separable into somatic and reproductive individuals than in the somewhat later stages. At just what stage in embryonic development (if this expression is permissible) the separation first takes place I have not been fully able to ascertain. Despite the number of coenobia at my disposal I found none showing the exact stage desired. Evidently, however, the differentiation of the reproductive cells must begin at about the 64-celled stage; if not, certainly at the next step. This brings us directly to the conclusion that we have in this simple *Volvox* aggregate a perfect example of the continuity of germ cells. It is in emphasis of this that I have named the species after the celebrated advocate of this conception.

Correlated, no doubt, with this early setting apart of reproductive cells is another interesting fact of their almost mathematically regular arrangement. Despite the fact that their numbers vary, occasionally running to high limits, yet even most of the variations are reducible to regular deficiencies of certain definite cells, on the one hand, or to equally regular additions or multiplications on the other. The phenomena remind one strikingly of the regularity of cell arrangement resulting from the segmentation of the ovum in higher organisms. The commonest numbers of primary sex cells are eight and ten, the latter predominating. The arrangement of the eight cells is as simple as possible (figs. 45 and 48), four, distributed at equal distances from each other, lie in one plane, well toward the posterior pole of the colony. The final closure of the young colony is always at this pole between these four cells. Situated at a considerable distance above these four cells lies the other four. They are invariably on a plane considerably above the equator of the colony and are so placed as to alternate with the lower four (note again figs. 45 and 48), cell for cell. I do not find an instance where the cells in the upper plane stand directly above those below, although they soon come to be so far above them that no reason is discernible for the special alternating arrangement. The ten-celled grouping repeats exactly that of the eight-celled, with the addition, however, of two more reproductive cells, in a pair, placed still nearer the vegetative pole. These cells are very peculiar.

To begin with, in the younger colony at least, they seem never to reach the size of the other cells; they are uniformly smaller, a difference which is frequently, though not always, maintained throughout their entire subsequent development. I am unaware of any other species of *Volvox* showing thus a regular discrepancy in the size of its sex cells. Again these two smaller reproductive cells are arranged in a definite manner with regard to those in the series just above them; they are not in a position of perfect symmetry, or only assume this position later. They lie almost touching two of the cells in the series just above them. The assumption of this position is again due, it would seem, to the position of the quadrangular or diamond-shaped opening of the unclosed colony; it is so large in nearly all cases as to thrust the smaller pair of cells out of their natural situation (see fig. 51). These eight and ten-celled groupings constitute by far the major number in the material which I have studied, and they also constitute the basis for all of the deviations. To begin with, the most frequent of these are due to the omission of one of the reproductive cells in any one of the series. Most frequently of all, one of the two smaller is wanting. But it is surprising to note that neither its mate nor any of the other cells are thereby shifted in their positions or altered in number or character. A single member is simply dropped out. Other deviations consist in interpolations and duplications or divisions. I have never found a single cell added on an old plane, making five in one circle, although nothing is commoner in other species. When cells are added they are added in pairs. Thus the two lower cells may rarely become four, all of the same small size which characterizes the lower two; much more frequently another pair is introduced, of full size, on a new level, as between the two main series. Rarely the cells of the lower four are shifted out of one plane, so that all, except the upper circle, appear plainly in pairs (fig. 39). Not only may we have these interpolations, but not infrequently each single cell in all the series has divided, as shown by the fact that in the young colonies these cells are but half the size of those in sister coenobia with ordinary counts (fig. 47); the two or four smaller cells at the base of the colony, however, may or may not divide with the rest. In very rare instances

both interpolation of series and division of members has taken place, giving surprisingly high numbers, which, so far as my observation goes, then always become ova.

This introduces another point which interested me much, *viz.*, the study of the destination of the different groups of cells. Such study showed that the definite arrangement applied equally to all. It is maintained with singular beauty in colonies which develop ova only, as figures 36 and 38 will show to a considerable extent, although the difficulties of flattening these colonies sufficiently for photography without distorting the symmetry of arrangement in their reproductive bodies is discouragingly great. A special tendency is plainly marked for the two or four smaller, posterior cells to develop into ova when the others do not (figs. 38, 42, and 43), while the opposite case I have never seen. Yet this latter is the only invariable rule that may be laid down. Otherwise every possible combination occurs. Any reproductive cell in any part of the colony may become either an ovum, a parthenogenetic colony of the ordinary type, or one of the dwarf male colonies later to be described. As above stated the regular arrangement tends strongly to be preserved throughout development, although it may finally become partially lost and is much less noticeable when the smaller reproductive bodies develop into large daughter coenobia. Yet figure 57, despite some displacement in the vigorous flattening necessary for photography, shows a beautifully typical arrangement, the two minor cells having developed into correspondingly small vegetative daughters. Rarely is the discrepancy in size so great, though seldom wholly absent. All in all, this definiteness in arrangement, yet almost entire indeterminateness in development, shows in this, as in no other species, the almost perfect parity of the different classes of primary reproductive cells. In no other species is this parity so great, although as I have suggested in my previous study, it is strongly indicated in *V. spermatosphara*. With these species before us it becomes, I think, impossible to deny the perfect homology of all the primary reproductive cells of the genus. And, if so, Klein's elaborate argument to the contrary; his refusal to admit the applicability of the conception of parthenogenesis, which Bütschli had applied to the asexually developing progeny; his desire

to homologize these asexually reproductive cells with the zoospores of the algae,—all these contentions fall to the ground. This will appear still plainer as we proceed, and especially at the close.

Touching next on the ova, we find them marked, in this species, by considerably greater size than in any other. Unfortunately, in the entire mass of material, none seemed to have been fertilized and I cannot state the nature of the oosperm wall. The unfertilized eggs, however, will be seen (figs. 36 and 38), although their maximum development may still not have been reached, to be larger than the zygotes in large female colonies of *V. spermatosphara* (fig. 4). I have not measured the very largest observed, but turning again to my slides, I readily find examples over 90 μ in their greater diameter. A further peculiarity lies in the fact that the ova are not spherical when seen from the side, so to speak. They are slightly flattened against the wall of the colony (fig. 36) just as are, in this species, the young colonies. This may be due to the action of the preservative (formalin), but it probably is not. Unfertilized ova of other species are not thus flattened by the reagent.

Finally, the mode of sperm-cell production in *V. weismannia* is also unique, though showing relationship to several other types. It is by means of dwarf male colonies which ripen their sperms before they are liberated, and indeed, probably lead but a brief and ineffectual motile life after they are freed from the parental organism. The species was but just entering upon a full period of sexual reproduction at the time the collection was made. An unlimited amount of material was therefore not at my disposal. Nevertheless, I examined, in one series, 131 parental coenobia bearing young male colonies in some easily recognizable stage of development (plate iv, figs. 46, 55, 57, 58, 59, 60). In all these the process bore evidence of striking uniformity even in minor details, the only exception being in the number of such dwarf colonies formed in one parental coenobium. This varies from one to ten, as the figures will show, the difference being largely due to the fact that usually a part of the primary reproductive cells develop into vegetative daughters instead. Figure 57 shows the only instance found by me in which all of the reproductive cells had given rise to male progeny. Among the oldest coenobia I did not find the male colo-

nies side by side with ova, but they were so in the somewhat younger ones. Indeed, as I have already indicated, nearly all combinations occur. Thus on plate III, figure 39 shows a colony in which all the progeny are ordinary vegetative daughters save one of the lower, the only one present of the lower pair, which is a young male. In figure 35 one of the lower pair and one of the first four are dwarf male colonies; in figure 41 one of the upper four only, and in figure 42 one of the lower four only. I shall return to this matter of the distribution of reproductive content, giving a series of concrete cases, as soon as I have described other phenomena connected therewith.

The presence of these dwarf male colonies, with accelerated sperm production, at once suggests a resemblance, if not a relationship, between this species and the alleged variety of *V. aureus* described by Klein,¹ as well as to *V. tertius*. Both of these forms showed the development of ripe sperms in daughter colonies before their birth. The resemblance is thus a real one, and a less close one exists to *V. spermatosphara* even, the sperm spheres, without all somatic cells, being, perhaps, comparable to these dwarf male colonies with but few somatic cells. The differences in detail are, however, very marked and very constant. Thus, in this species the dwarf male colonies are not paralleled, as in the European forms, by dwarf female colonies. Although the egg cells are set apart never so early in *V. weismannia*, they grow slowly and mature only in colonies that have long been free. The structure, too, of these precocious male colonies is unique. Thus the number of somatic cells, in proportion to the spermogonia, is surprisingly few, though it by no means varies to especial closeness to the sperm spheres of *V. spermatosphara* (see especially pl. iv, fig. 60). The number of sperms in the bundles is again quite beyond that in any other *Volvox* save the unrelated forms of *V. globator* and its American ally, *viz.*, 64 and 128. In every instance observed by me these two sizes occur side by side, and, curiously enough, in about the same proportions, the larger size always much the most numerous. Even in the younger colonies with undivided spermo-

¹Ludwig Klein: Neue Beiträge zur Kenntniss der Gattung Volvox. Berichte der Deutschen Botanischen Gesellschaft, VII: 42, etc.

gonia, of which I observed a great many, these latter cells were always of the same two distinct sizes and the larger always much the more numerous. These facts can, with care, be made out in figures 58 and 59. Both the position and the shape of the sperm bundles is also peculiar. They lie well within the colony, leaving the mere smattering of somatic cells to form the entire peripheral contents (fig. 60). Even the spermatogonia take up this position some time before their final divisions. The process is just beginning in figure 59 and is carried further in figure 58. In all other species heretofore described the bundles of sperms are represented as quite flat in their final form; here they are all of them slightly concave (fig. 61). The sperms, too, while showing the terminal cilia and general form of the *aureus*-type, are more slender than in any other species except *V. globator*.

The above may be considered a fair resumé of the most important characters of this species so far as they are peculiar and so far as they could be ascertained from the material at my disposal. One other character, however, was very evident in the material and was so new and strange that I at first thought it peculiar. I have, however, since discovered it occasionally in other species. And in all it remains the most interesting phenomenon which I have thus far met with in the genus. I refer to the invagination and, finally, the complete inversion, or turning inside out, of many of the young colonies. The process begins at various periods before the closing of the young colony and is finally completed by a reclosure of the colony, the surfaces of which are now reversed, at a somewhat later period. I have seen no allusion to similar observations by other students of the genus. This fact, together with the bizarre nature of the phenomenon itself, led me to be very doubtful as to its cause. The possibility suggested itself that the whole process might be due to some unwonted effect of the five per cent formalin used as a preservative. Yet this usually conserves well the general shape of colonies, both young and old. In any case I deemed the process peculiar to *V. weismannia*, and that if not due to the action of the formalin, it might well be a pathological result of growth at too high a temperature. The volvox collection in question had been taken in water that "felt warm to the hand." One

after another, however, each of these conjectures was disproven. I at last found young colonies of *V. spermatosphara* which were also undergoing the process at the time of their preservation. While, even in my earliest collections of living material, in July, I succeeded in detecting a few instances, not one of which showed any other sign of abnormality. Later in the season I met with them, never in great numbers, but from time to time, in each of the species which I collected. They occurred, too, in freshly collected material brought from near-by sources with the utmost care. Last of all, I found the same thing, in at least one instance, in a colony of the large *globator*-like species next to be described, which I took from relatively cold spring water. I judge the phenomenon, then, to be not wholly abnormal. I regret that I have in no case been able to retain and watch the development of the inverted colonies. I have only watched them through a few stages of the process.

An examination of figures 30, 31, and 32 will show how conspicuous are the young colonies of *V. weismannia* which result from this inversion. A superficial glance at even unstained material revealed the astounding presence of the primary sex cells on the outside of young colonies. Sometimes one, sometimes several, and, now and then, all of the young daughters in the half-grown coenobia were in this condition. Unfortunately the oldest coenobia with still larger daughters did not contain them, and I was accordingly unable to trace their development beyond about the time of the reclosure of the inverted progeny. I was not able to photograph the earlier stages of the transformation very clearly, but figure 22, from *V. spermatosphara*, and figures 25, 27, 28, 29, and 33, from *V. weismannia*, suffice to make apparent most of its stages. The young colonies, at the beginning of the process, lie with the unclosed pole directed toward the center of the coenobium. The closed or anterior pole, lying against the wall of the parent, folds inward, exactly as in a true case of gastrula formation. When, however, the invagination is completed (fig. 22), the process does not pause, the opening at the posterior pole yields to the pressure of the opposite infolding layer; it is stretched, though apparently not torn asunder, and the anterior pole of the young colony presses quite

through the open posterior pole. An intermediate stage in the process at which it seems to slacken somewhat, and is therefore quite frequently found, is shown in figures 25 and 32. It looks exactly like a rolled up child's cap. Soon, however, the crown is pressed quite through the rim and a form is reached resembling a wide vase or an old time kettle with flaring rim (fig. 27). This then rapidly closes, the whole assuming the original form of a young colony save for indentations or pockets which are soon formed under the reproductive cells (figs. 30, 31, and 32). The ova which show but indistinctly in the inverting colonies as figured, are, in reality, very conspicuous in shifting foci. So loosely are they held to their points of anchorage during the inversion, it seemed at first probable that they might frequently be quite dislodged, I was even inclined to interpret certain loosely scattered cells of appropriate size found in the matrix of the parental coenobium as possibly of this origin. Further observations did not bear this out, however. The reproductive cells are not loosened during the inversion; and the scattered cells in the old colony proved to be chlorophyll-containing flagellate parasites, which penetrate the matrix from without and there undergo a further development. The reproductive cells of the young inverted colonies meanwhile, loosely held at first, rapidly sink into little depressions, well shown in figure 32. I have seen none that were sunk deeper or gave signs of re-entering the colony; but, in the material at hand, hundreds of young colonies were present, with the eight and ten reproductive cells respectively, looking, according as seen from one pole or the other, exactly like figures 31 and 32.

Seeing the abundance of these forms, and noting considerable variation in the developmental stage at which the process begins, I sought for instances of invagination in young but completely closed colonies. I half feared I should find such, and thus unwittingly give occasion for some younger Haeckelian philosopher to load our delicate *Volvox* with a yet more ponderous weight of phylogenetic fancy. Fortunately, however, no such "true gastrulae" could be found. Slightly cupped individuals were common enough, but none deeply invaginated, so we need not on account of this new phenomenon dream yet further of *Volvox* as the primal metazoon. The real

interest in the process lies in its possibilities as, so to speak, a natural experiment. The reproductive cells, which have originally three distinct modes of development, are, in mid-career, suddenly placed in a new yet evidently not fatal environment. Will this introduce into the already complex life-cycle of this species yet other and varied links? The single though copious collection of material, which is all I have of this species, does not even suggest an answer. I should be much pleased if others should be so fortunate as to find the complementary phenomena.

Having described the reproductive phenomena of this species, it may be worth while to indicate briefly the actual contents of several coenobia taken almost at random from the slides. This will substantiate, to some extent, the foregoing statements as well as supplement the meager representation in the figures.

Number 1. Coenobium with content of 10 bodies; upper row of four daughters, each with eight reproductive cells, two inverted, two typical; second row, one typical daughter with eight reproductive cells, two with high numbers (18 or 20) and one in process of inversion with eight or ten reproductive cells; two lower members of the content remaining as true ova.

Number 2. Large coenobium with content of ten daughters; the first and second series, with four in each, all typical daughters with eight reproductive cells each; the two lower members also daughter coenobia but smaller, one with eight, the other with sixteen reproductive cells of half the size. No inversion.

Number 3. Smaller coenobium with content of eight bodies; all of upper series and two of lower are very young daughters, seemingly all with eight reproductive cells; remaining two in lower series, large ova.

Number 4. Content of ten bodies. Upper series: two typical daughters with 9 and 10 reproductive cells, respectively; remaining two, in process of inversion, show 8 or 10 reproductive cells. Second series: one typical daughter with 18 or 20 reproductive cells; one with but 6, and two completely inverted with each 8 reproductive cells. Lower pair of bodies remain as large ova.

Number 5. Coenobium with content of nine daughters. Upper series: four typical young colonies with 8 reproductive cells each.

Second series shows absence of one member, leaving but 3, each of which bears 8 reproductive cells; one completely inverted. Lower two members small daughters, both completely inverted, with 8 reproductive cells each.

Number 6. Coenobium with content of ten daughters. Each member in the two upper series a typical young colony with 10 reproductive cells. The lower two, much smaller, with 8 reproductive cells each, one completely reversed and one in process.

Number 7. Coenobium with content of ten bodies, all ova except one of the upper series, which is a young dwarf male colony.

Number 8. Coenobium with content of eight bodies. Upper series: 2 large ova, one typical and one inverted daughter, each with 8 reproductive cells. Lower series: three large ova and one young dwarf male colony.

Number 9. Coenobium with content of ten bodies. Upper series: all young daughters with 8 reproductive cells, of which two are completely inverted, one in mid process and one just beginning. Second series: likewise all daughters with 8 reproductive cells each, of which one is typical and 3 are completely inverted. Lower two members: one an inverted daughter with 8 reproductive cells, the other a young dwarf male colony.

Number 10. Coenobium with content of ten bodies. First and second series each contain 4 typical daughters with 8 reproductive cells each. Lower two members both ova.

Number 11. Coenobium with content of ten bodies. First and second series composed of typical daughters with 8 or 10 reproductive cells each, save one member in upper series which has a much higher number too difficult to make out. Two lower members are very small inverted daughters, each with 8 reproductive cells.

Number 12. Coenobium with content of ten bodies, all typical daughters with 8 reproductive cells each, except the lower pair, one of which is an ovum and one a young dwarf male colony.

VOLVOX PERGLOBATOR

I little thought to hit upon yet another distinct species of the genus. I had indeed several years ago seen a few colonies of a large *Volvox* closely resembling *V. globator* save that a very few

female colonies showed a wholly unwonted number of ova or zygotes. This suggested at least marked variation from the European type. Hardly had I found my first *Volvox* pool this season, however, than I hit upon this type, the first time I had ever seen it alive or in any quantity. It proved to be a most rampant grower in the writer's locality (Lincoln, Neb.). After a heavy rain in mid-August ten days sufficed to produce, multiply and bring to copious reproductive phases an amount of material which almost thickened the water in tiny depressions about the border of a weedy pool on low, soggy ground. When sexual reproduction was at its height the reproductive colonies, especially the females, lay literally in handfuls at the bottom of such cavities. Even a cursory examination of such material showed it to be specifically distinct from the European *V. globator* as this has been described and figured. Moreover, this latter species has not been considered of a highly variable nature. As I have not had time to prepare plates of this species, I will in the present article but note its more obvious specific differences.

To begin with, it grows in this locality to just about double the size ascribed to *V. globator*. The maxima given for this latter species have been from 500 to 800 μ for the vegetative colonies, the sexual colonies being usually, though it appears not always, smaller. *V. perglobator* always exceeds these dimensions in this locality, even when the conditions of excessively rapid multiplication and overcrowding should tend to reduce its size as much as possible. All of my collections showed vegetative colonies beyond 1 mm. in diameter, while 1200 to 1400 μ colonies were readily obtainable, and from a slightly larger pool I measured one old furrowed colony of nearly 1600 μ diameter. Even the female colonies from this source were, many of them, just about 1 mm. in diameter, sometimes exceeding this in the longer dimension.

The second prominent character separating this species from *V. globator* is its dioecious sexuality. Overton says of *V. globator* that all observers agree upon its monocious (hemaphrodite) character. Klein, it is true, in his monograph published simultaneously with Overton's, throws some doubt upon this assertion, deeming that *V. globator* "may yet be shown to possess more complicated sexual relations." This has not, to the writer's knowledge, yet been shown;

but, in any case the classic species must be chiefly hermaphrodite. Its American congener, on the other hand, in the large masses of sexually reproductive material that I have examined, shows uniform separation of the sexes. Among thousands of coenobia I did, indeed, find one enormous female colony, loaded with zygotes, which showed, on one side, a single well developed group of sperms, while near it a vacant space showed evidence of the escape of another. But a single instance among thousands indicates an abnormality only. The sperm masses in this species, seem, further, to always escape outward, into the surrounding water, never into the interior of the coenobium. Among material rich in male colonies these groups of free sperms may readily be found, swimming with vigorous independence among the various coenobia. Self-fertilization, therefore, which is asserted for *V. globator*, is obviously precluded here.

Not only is this species dioecious, but even the combination of sexual cells with parthenogenetic reproduction, spoken of as if not rare in *V. globator*, is here only an anomaly. I have seen but three such colonies.

Much more striking to the eye than any of the above characters is the number of reproductive cells which this superb species presents. Even in the most poorly developed material collected in the writer's locality, the number of ova, or oosperms, often exceeds one hundred, while in the finest material yet found it is not infrequently between three and four hundred, the large oval female coenobia being thickly studded with crenate zygotes, leaving only a small area of purely somatic cells at the anterior pole. When final counts are made, I think considerably higher numbers will be found. *V. globator*, on the other hand, shows as maxima, according to different observers, from thirty to fifty female cells.

The male colonies differ much in size, number, and manner of development. Sometimes they develop all their sperms nearly simultaneously; at others their development is spread over a very considerable period, nearly all stages being present at one time. Old colonies may be found almost devoid of sperms or anlagen of them, all or nearly all having been shed. Excepting such colonies as these, however, the numbers of sperm groups is always very high. In

place of the four or five of *V. globator*, we have, in the main, from 50 to 150 sperm masses or their anlagen in one colony.

Finally I will mention the most unique and most interesting character of the species, *viz.*, the manner of development and the final form of the sperm bundle or sperm group; for "bundle" it is not and still less a "platelet." I introduce the term "sperm globoid," for the single aggregate of sperms here, instead of being flat and plate-like is, in its formative period, a hollow, globular body, strangely simulating a minute *Volvox* colony. As the spermocytes become differentiated into perfect sperms this globular body becomes much flattened, retaining, however, its cavity and showing sperms with outwardly directed flagella on all sides. These latter do not project similarly from all the sperms; one side, even while the globoid is still within its parental lodgement, always shows the flagella parted, or, rather, radiating from a center; as we approach the periphery they bend around the sides and extend straight backward, as do all those on what we may call the posterior side. The whole arrangement is obviously an adaptation to a special mode of locomotion, a rapid spiral rotary movement with one pole habitually directed foremost.

I have not as yet sufficiently studied the form of the single sperms. They are obviously shorter and smaller than any I have seen before, and are very numerous. I think a single globoid probably contains twice the maximum number hitherto given for the single sperm platelet in any species. I find that these sperm globoids are subject to some variation, which I hope to study in detail later. I will but mention now that in material grown in much less sunny climates, as Maine and Michigan, although undoubtedly of this species, the globoids do not become always complete. The form is quite the same. But a small, round opening remains on one side. This suggests the possibility of a variation so considerable in amount as to link this species with the typical *V. globator*. There is little prospect, I think, however, of such extensive variation, more especially as *V. globator* has shown itself a rather non-variable species.

As to the minor characters of the vegetative cells., etc., they plainly coincide, in the main, closely with those of *V. globator*. The excessively numerous, highly stellate cells often quite duplicate the

appearances of the figures of Overton, Meyer, etc. In the oldest vegetative coenobia the appearance is a great exaggeration of anything hitherto described. The single cells, becoming far removed from each other, extend their cytoplasmic processes in irregular, bent lines, until the cell body is hardly noticeable, and the appearance, under a moderate magnification, is that of a sponge-like reticulum. As mentioned earlier in this paper, I was able to verify perfectly the extremely delicate connecting fibrils which Meyer showed were the real connection of the cytoplasmic processes one with another. But in a few cases I observed, in old colonies, isolated instances of a very different type of union. The heavy cytoplasmic processes had themselves quite penetrated the bounding surfaces of the gelatinous envelopes and united pairs of cells in a wholly different manner. The connection in rare cases had been strengthened until two cells had entered into close plastogamic union, quite resembling that seen among rhizopods; the diameter of the connecting bridge being little less than that of the individual cell bodies. Such cases resembled binucleate or dividing cells, save that the age of the colony and the size of the cells precluded such interpretation.

In distribution, *Volvox perglobator* is probably little less than universal in the United States. The most frequent species in my own locality, I have also found it in the arid sand-hill region of western Nebraska. Although not quite certainly established, vegetative colonies probably belonging to this species are found in material from Washington. It occurred sparingly in the collection from Rocheport, Missouri, and was well demonstrated and abundant in plankton from ponds near New Orleans. I have seen the same species from St. Louis, Missouri; from the vicinity of Ann Arbor, Michigan; probably from Massachusetts; and, finally, from the neighborhood of Sebago Lake, Maine.

Undoubtedly this is the species most frequently called *V. globator* in America. But *Volvox* would seem to have been more casually studied than well nigh any other organism of interest. Figures have been published and labeled *V. globator* that bore no resemblance to the description or figures of European writers; even vegetative coenobia bearing young colonies of thousands of cells each have been mistaken and figured for colonies containing ova. All of

which shows the need of more careful and extended work. Whether the European *V. globator* really occurs with us at all or not, I have as yet no exact evidence to determine. Very probably it does, though less abundantly.

GENERAL REMARKS

In conclusion it is worth pointing out that these new species of *Volvox* raise or throw light on several interesting problems with regard to the genus. Without attempting full treatment, a little space may be devoted to each.

The first of these is the nature of the *Volvox* aggregate as such: what value has it over and above the unicellular condition, or that of the much simpler aggregates in the same family. The answer has been given that it is an "Ernährungsgenossenschaft," a nutritive society. The evidence for this has lain mainly in the fact of the cytoplasmic connectives uniting cell with cell, and especially their increased number in *V. aureus* at the points of union between reproductive and somatic cells. The further fact has been cited that division and growth of the somatic cells is not continued after the development of the reproductive cells has begun. Now a rapid survey of the facts recorded for *V. spermatosphara* and *V. weismannia* will show, I think, that these positions are not well taken. The first species possesses no connectives between the cells whatever, if my observations are correct. Its reproductive cells slip below the surface at a very early stage in their development and seem to rest only in the slightest contact with the somatic cells. In *V. weismannia* I am less certain about the general fact of connectives, but they are probably absent, and the case of the reproductive cells is much more convincing. Here their advent does not coincide with the cessation in division of the somatics, and the development of both is for a long time simultaneous. Especially instructive in this connection is also the development of the sperm spheres and the dwarf male colonies of the two species respectively. In the case of the former a large number of germ cells develop rapidly without the immediate proximity of any somatic cells whatever. In the case of the latter the somatic cells are small and utterly insignificant in number, while the germ cells are rapidly developed and numerous, producing sperm bundles of unusual size. Such instances seem an absolute disproof

of the value commonly assigned to the cytoplasmic connection as found in *V. aureus* and *V. globator*. I may add the observation which I have more than once made that in instances where young colonies within the parent have been almost completely destroyed by rotifers, leaving a part of their reproductive cells with little or no somatic support, these cells have none the less continued to develop and even exceeded in size the reproductive cells of sister coenobia within the same parent which had not been injured. If, then, we are to regard the *Volvox* aggregate as a communistic nutritive society, the medium of nutritive transfer must be, not the cytoplasmic processes, but the gelatinous matrix itself and this alone. Even this, however, is rendered improbable by such facts of reproduction as I have cited in my previous article in regard to the "second form" of *Volvox* there described. The life of the cells in *Volvox* is probably nearly independent, that of the reproductive cells with the rest; they doubtless grow mainly by their own nutrition, their chlorophyll being always abundant as long as growth continues. Protection, and locomotion, leading to suitable illumination, are doubtless their only advantages.

Upon another general matter of still greater interest I will, for the present, touch with but a word. The determination of sex is a matter of great present interest. Complex facts and complex interpretations seem tending to supplant the simple nutritive hypothesis that for a time was deemed of universal application. Sex in *Volvox* is almost at its beginning, yet it is universally conceded. The relations, moreover, are anything but simple; parthenogenetic egg cells developing into colonies of like content; or into male colonies, or female colonies; or into colonies of various mixed types. Even in a single species the phenomena are involved. Yet just herein lies, perhaps, the possibility of tracing cause and effect and arriving at a general conclusion. The different species, moreover, offer altogether varying degrees of advantage as subjects for analysis. Without attempting, at the present time, a restatement of the facts with this end in view, the writer will but remark that the detailed phenomena of reproduction in *V. spermatosphara* and still more in *V. weismannia* seems to yield strong support to the hypothesis that nutritive causes, manifesting themselves especially in the size of the

cells, are an important factor in determining the sex of the reproductive cells or their products in these incipient multicellular organisms.

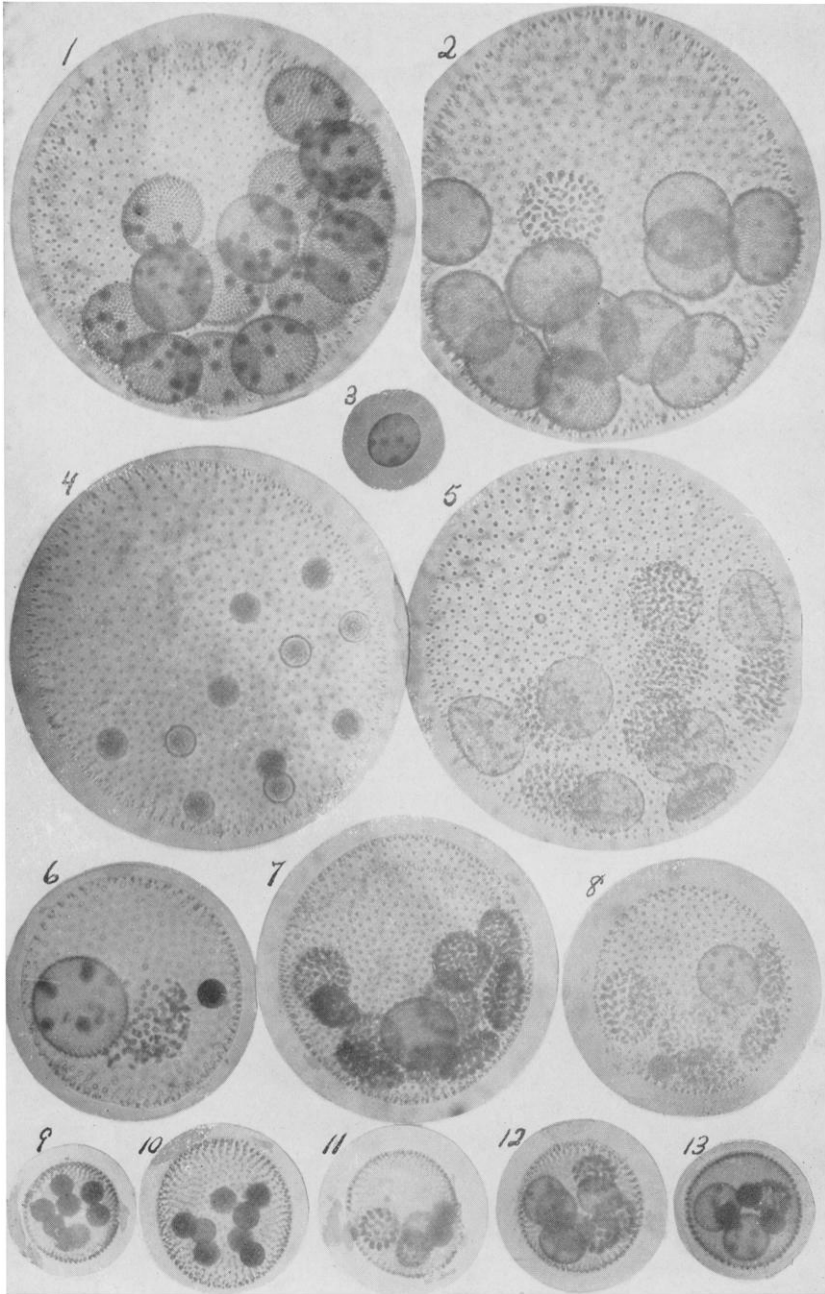
Next as to the general interpretation to be placed upon the peculiar mode of sperm formation in *V. spermatosphara* and *V. weismannia*. I have said of the sperm spheres that they may be interpreted either as reduced colonies in which somatic cells have been completely replaced by reproductive members, or as mere groups of multiplied spermogonia which, coincident with the general habit of all growing cell aggregates of the genus, have assumed a spherical form. The dwarf male colonies of *V. weismannia* might well tend to support the first hypothesis of simple, reduced, precociously reproductive coenobia. But again the ontogeny of these dwarf male colonies reduces this probability. For these dwarf male progeny are distinct from almost their earliest segmentation, every stage of their development being peculiar to themselves. While seeking out these facts it suddenly occurred to me that another hypothesis was possible, and, indeed, far more probable than either of those mentioned, *viz.*, that both the sperm sphere and the dwarf male colony represent, not secondary acquisitions of any type, but earlier phylogenetic stages in the evolution of the genus, preserved in these species on the male side, so to speak, only. In my former article I spoke of the striking resemblance between a sperm sphere in the spermogonia stage and a *Eudorina* colony. And since the finding of numerous sperm spheres with but thirty-two members the parallelism becomes still closer. We might even, without violence, assume the original development of *Volvox* to have taken place directly from *Eudorina*, the sperm sphere serving as an almost perfect connection between the two. Colonies of *Eudorina* have been reported in which every cell became a platelet of sperms. The only change to the sperm sphere would be the loss of independence, a possible increase in size, reduction of cilia, etc., all minor changes. The dwarf male colonies would evidently represent a slightly higher stage in the evolution toward the *Volvox* aggregate, *viz.*, the splitting up of a few cells, in the early time of segmentation into somatic members, while the majority develop as before. On the female and ordinary parthenogenetic sides the species have obviously reached a much higher plane.

If the foregoing interpretation is correct it suggests at once an arrangement of the known species of the genus in a more or less perfect series. *V. spermatosphara* stands lowest, not only because of its *Eudorina*-like sperm spheres, but because of its lack of cytoplasmic cell connections, its small size, etc. Just above it stands *V. weismannia*; above this undoubtedly *V. tertius* and the peculiar form (very probably a distinct species) described by Klein as a variation of *V. aureus*. Possibly, indeed, this form, when fully studied, may rank yet lower in the series. Considerably above these, at least in its ordinary types, stands *V. aureus (minor)*; while yet further removed stands *V. globator*, with its late maturity and reproductive cells which seem to be derived directly from somatic cells which have already reached a high degree of differentiation. Highest of all stands *V. perglobator*, which seems to intensify or carry further nearly every feature of its better known relative. The sperm globoids of this species might seem possibly open to an opposite interpretation, as primitive sperm aggregates that retained the form of a complete colony. The form of the ordinary sperm platelet is, however, as long since pointed out, a still more primitive phylogenetic form, and the sperm globoid of *V. perglobator* undoubtedly represents a later hypertrophy, so to speak, of the simpler and more common type.

Be these interpretations, however, as they may, the little genus *Volvox* is evidently rich in interesting surprises, and this paper will quite have served its purpose if it stimulates their discovery as well as aiding, in some degree, the general study of our American forms.

In conclusion I wish to thank the following persons, from whom I have received valuable collections of material, greatly aiding me in the present study: Prof. R. H. Wolcott, Lincoln, Neb.; Dean H. B. Ward, Lincoln, Neb.; Dr. Elda R. Walker, Lincoln, Neb.; E. Foster, New Orleans, La.; Bertram G. Smith, Syracuse, N. Y.

PLATE XXIII



EXPLANATION OF PLATES

Plate XXIII

Volvox spermatosphara

The figures on this plate give a typical representation of the forms, sizes, and content of the different colonies of the species. All magnified about 79 diameters.

FIG. 1. Medium to large coenobium containing 14 typical daughters.

FIG. 2. Medium to large coenobium containing 11 rather large daughters and one sperm sphere composed of platelets of developed sperms.

FIG. 3. A free coenobium showing approximately the smallest size at which they escape from the parent.

FIG. 4. Large female coenobium containing 11 oosperms.

FIG. 5. Medium to large coenobium contains six daughter coenobia and six mature sperm spheres. Several of the daughters are somewhat distorted.

FIG. 6. Coenobium containing one daughter, one young oosperm and one sperm sphere disintegrating into its component sperm bundles in the manner invariable in this species.

FIG. 7. Coenobium containing 10 nearly mature sperm spheres and one daughter coenobium.

FIG. 8. Coenobium containing seven nearly mature sperm spheres, one young oosperm and one daughter coenobium.

FIGS. 9 AND 10. Small female coenobia (very characteristic) containing eight ova each.

FIG. 11. Small coenobium containing three immature (two in spermatogonia stage) sperm spheres and two ova as young oosperms. This combination is by far less common than any of the others on the plate with the possible exception of the balanced coenobia in figures 5 and 12.

FIG. 12. Small coenobium containing four daughter coenobia and four immature sperm spheres.

FIG. 13. Small coenobium with two daughters, one immature sperm sphere and three ova.

Plate XXIV

The figures in the upper portion of plate show more eccentric coenobia of *V. spermatosphara*; the smaller ones toward the lower end show the phenomena of inversion in young coenobia of *V. weismannia*.

FIG. 14. *V. spermatosphara*, small, containing one sperm sphere of 32 sperm bundles, and one ordinary daughter. $\times 79$.

FIG. 15. Small sterile coenobium of same species. $\times 79$.

FIG. 16. Coenobium with six daughters and one sperm sphere, the platelets of which contain 64 sperms each. Very rare. $\times 79$.

FIG. 17. Small coenobium with one relatively large daughter and one ovum or young oosperm. The latter is *not* in the daughter, but is below it pressed against its wall. $\times 79$.

FIG. 18. Small coenobium containing mature sperm sphere only. $\times 79$.

FIG. 19. Medium to large coenobium containing but single daughter. $\times 79$.

FIG. 20. Coenobium showing maximum amount of crowding by daughter coenobia, of which there are 10, only seven or eight showing in the single focus. $\times 79$.

FIG. 21. Smaller coenobium showing single daughter of relatively large size. $\times 79$.

FIG. 22. Detail from one side of a young coenobium of *V. spermatosphara*. The young daughter coenobia still show no sex cells. One of them is still unclosed and displays well the process of invagination, the first stage in inversion. The daughter coenobium beside it, still not quite closed, is perhaps beginning the same process, being flattened on the side toward the parental wall. $\times 217$.

FIG. 23. Coenobium of *V. spermatosphara* with four daughters, showing moderate degree of crowding. $\times 79$.

FIG. 24. Detail from one of two coenobia found representing possible connecting types between *V. spermatosphara* and some other species. The figure shows, mainly, a sphere or young colony made up almost entirely of sperm platelets of 16 sperms each, a few undivided and therefore possibly somatic cells being among them. It is hard to separate these, in the figure, from the cells of the parental coenobium, but this may be done near the periphery. $\times 217$.

FIG. 25. Young daughter coenobium of *V. weismannia* in process of inversion. Three of the sex cells are dimly visible. $\times 319$.

FIG. 26. Coenobium of *V. spermatosphara* with four daughters and one mature sperm sphere; shows crowding and crushing of the less resistant sperm sphere. $\times 79$.

FIG. 27. Nearly inverted daughter of *V. weismannia* in the "kettle-shaped stage," the loosely attached reproductive cells show but poorly at the sides. $\times 217$.

PLATE XXIV

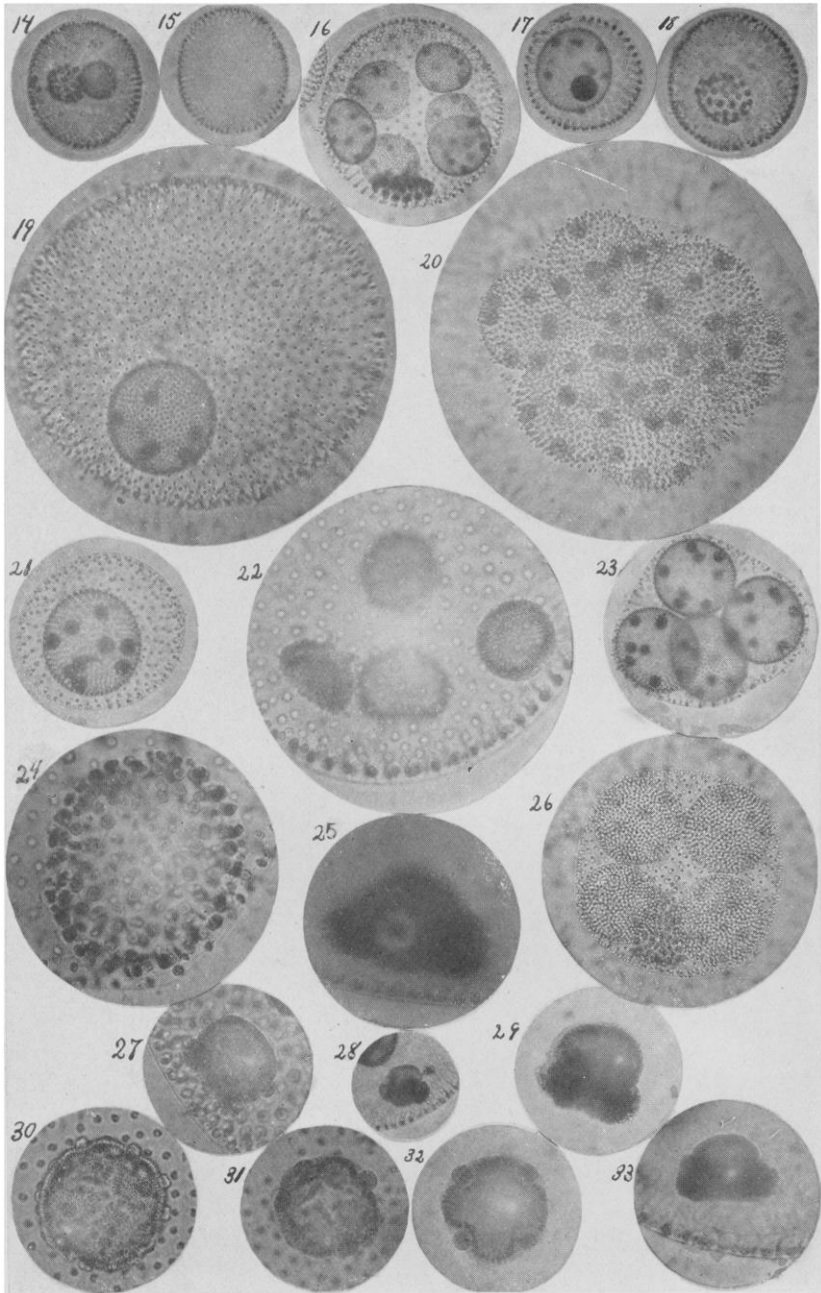


PLATE XXV

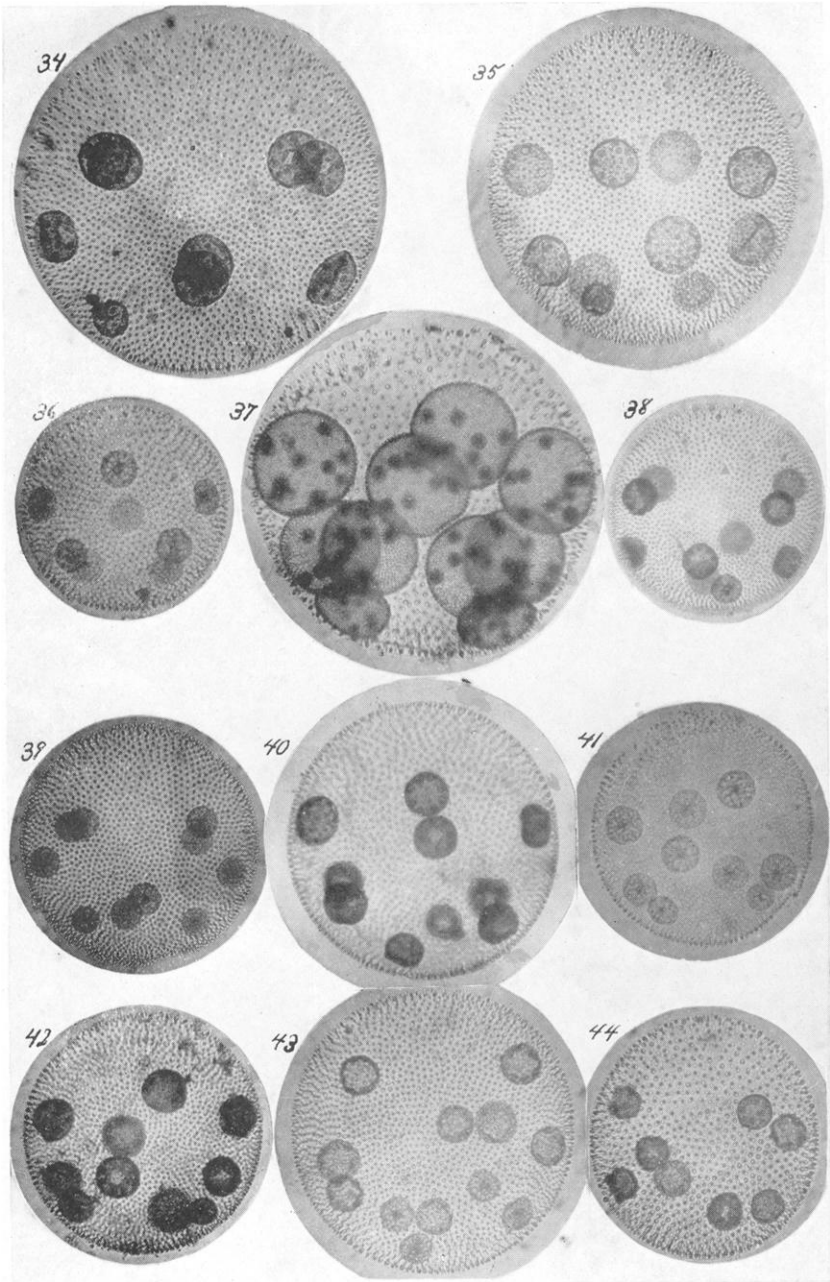


FIG. 28. Inversion of very small and young coenobium of *V. weismannia*, at the earliest stage found; the reproductive cells are present but hard to make out in figure. $\times 217$.

FIG. 29. Another instance of inversion in same species; young colony removed from parent. $\times 217$.

FIG. 30. Completely inverted daughter—same species—about to reclose, as shown by the x-shaped cleft in the middle. The reproductive cells are here numerous, probably 18 or 20, and are plainly visible. They have but just begun to sink into the depressions which form beneath them.

FIGS 31 AND 32. Small daughter coenobia of *V. weismannia*, seen from opposite poles, one removed from parent before photographing. Each has eight reproductive cells, those in figure 32 having sunken well into the pits that receive them.

FIG. 33. Another, rather early, stage in the same process and same species.

Plate XXV

Volvox weismannia

All magnified about 79 diameters.

FIG. 34. Coenobium with nine daughters typically arranged. The lower one lacks a mate that would have made the typical number 10. The lower daughter is typically smaller; it is completely inverted, showing reproductive cells on outside. The other daughters are still unclosed, showing the quadrangular openings on one side in several instances.

FIG. 35. Typical coenobium with 10 progeny, all of upper series with 10 reproductive cells each. Of second series, three are typical, while one is a young male colony. Lower two show one small inverted colony and one young male colony.

FIG. 36. A typical female coenobium with eight ova. The regular arrangement is fairly well preserved; note flattened form of ova when seen from side.

FIG. 37. Medium-sized, nearly mature coenobium with 10 daughters, the regular arrangement being nearly preserved. The reduction in size of the two lower members is somewhat greater than usual.

FIG. 38. Young coenobium with 10 reproductive bodies showing typical arrangement. One of the two lower is an ovum. The one of the lower four toward the left is in the process of inversion.

FIG. 39. Female coenobium with 10 ova; regular arrangement well preserved. The arrangement is, however, not typical, in that only the upper four ova are in one plane.

FIG. 40. Coenobium with 10 reproductive members, of which one in upper series (left hand) is becoming a young male. In the second plane

the two toward the right are both inverted, as is also the farther of the two lower ones.

FIG. 41. Female coenobium with nine ova, one in the upper series wanting. The ova are considerably displaced in the flattening of the whole colony but the arrangement can still be discerned, especially by the two lower and smaller members.

FIG. 42. Coenobium with 10 reproductive members, somewhat displaced. The two lower are remaining as ova, and one of the lower four is a young male colony.

FIG. 43. Coenobium containing the less frequent number of 12 reproductive members. Unfortunately the originally regular arrangement is much disturbed, but can be made out. The four lower members are undisturbed in position and are all ova.

FIG. 44. Coenobium with eight progeny, the arrangement considerably disturbed. All are typical save the left member in the lower series, which has just inverted, showing the "kettle stage."

Plate XXVI

Volvox weismannia

FIG. 45. Young coenobium (taken shortly after closure) seen from side, showing the typical oblate form peculiar to young of this species. The eight ova occupy typical positions. $\times 217$.

FIG. 46. Nearly mature coenobium with but seven progeny. Note, however, preservation of the typical positions, one member being represented by a noticeable vacancy in the lower series. One of the upper four is a dwarf male colony with spermogonia nearly ready for division. $\times 79$.

FIG. 47. Young daughter colony slightly older than figures 45, 48 and 49. It shows a less frequent instance, double the number of reproductive cells. Their position in pairs may be partially noted; also their size reduced by one-half. $\times 217$.

FIG. 48. Typical colony, with eight reproductive cells, seen from one pole. Compare with figure 45. $\times 217$.

FIG. 49. Typical young colony with 10 reproductive cells. The two slightly smaller cells, removed a little farther from the periphery, are the lower pair. $\times 217$.

FIGS. 50 AND 51. Still younger, unclosed, coenobia seen from opposite poles. Each has 10 reproductive cells. Note, in figure 50, the determination of the position of the smaller pair by the fold-like borders of the opening. $\times 217$.

FIG. 52. Early differentiation of reproductive and somatic cells.

FIG. 53. Like figure 50, but a stage younger.

PLATE XXVI

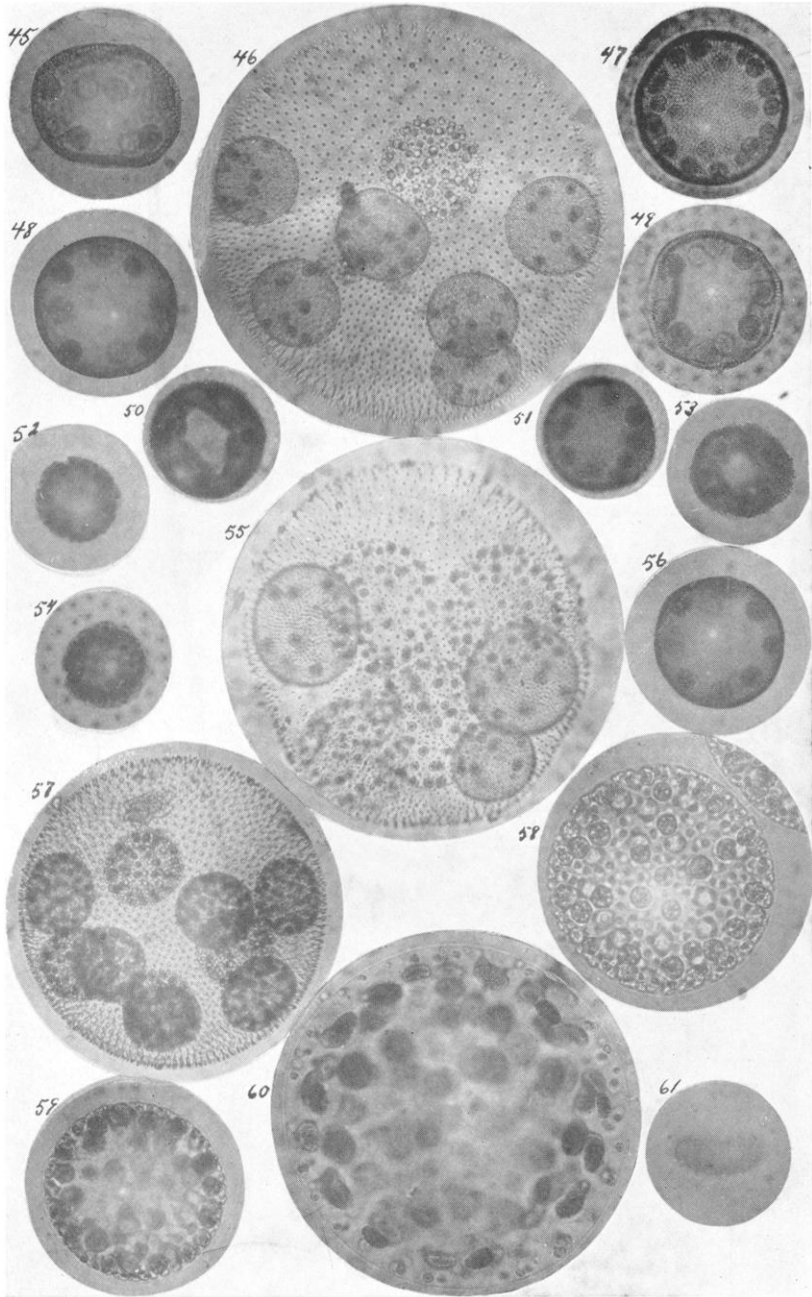


FIG. 54. Very young dwarf male colony showing (dimly) the very early differentiation of reproductive cells (spermogonia) and somatic cells.

FIG. 55. Nearly mature coenobium showing four dwarf male colonies, their spermogonia being just ready to divide. This coenobium was included in the plate partly as an example of irregular number in general content, *i. e.*, seven instead of eight young. Re-examination of the slide, however, shows fragments of another dwarf male colony which had been nearly destroyed by a rotifer, thus making the typical number eight. The small size of one of the lower four is, nevertheless, not usual in an assemblage of eight young. $\times 79$.

FIG. 56. Young coenobium much like that in figure 49; the lower pair of cells are dimly visible. $\times 217$.

FIG. 57. Coenobium, nearly mature, bearing ten young colonies, all dwarf males. Rather rare. $\times 79$.

FIG. 58. Immature male colony in the stage at which most of the spermogonia have taken up their final position within the colony, leaving the few somatic cells alone to constitute the peripheral series. $\times 217$.

FIG. 59. Like the preceding save at a somewhat earlier age, some of the spermogonia being still at the periphery of the sphere while others are receding below the somatics. $\times 217$.

FIG. 60. Mature dwarf male colony from within the parent, showing mature sperm bundles. The focus is at the periphery of the colony to show the few small somatic cells at the periphery while the mature sperm bundles (of two sizes) are well within, forming as it were a second layer. $\times 217$.

FIG. 61. Single sperm bundle of approximately 128 sperms (larger size) seen from side and showing the slightly concave form characteristic of this species.